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SPORE PRODUCTION OF MICRO-FUNGAL ISOLATES FROM THE N AND S FACING SLOPES OF EVOLUTION CANYON, LOWER NAHAL OREN, MT. CARMEL, HAIFA, ISRAEL

N. Rosenzweig & P.A. Volz

East Michigan Mycology Associates, 1805 Jackson Avenue, Ann Arbor, Michigan 48103-4039 U.S.A.

ABSTRACT

Eleven micro-fungal species isolated from both the North Facing Slope (NFS) and the South Facing Slope (SFS) of Evolution Canyon, Lower Nahal Oren, Mt. Carmel, Israel were examined for spore formation. Sporulation varied for the same species according to isolates recovered from the NFS, SFS, and carbon sources. *Mucor hiemalis* sexual zygospore production also was monitored.

KEY WORDS: Israel, micro-fungi, spore production, canyon slopes

INTRODUCTION

Much attention has been directed to the soil micro-fungi found in Evolution Canyon, Lower Nahal Oren, Mt. Carmel, Haifa, National Park in Israel. Variations in habitats are evident between the North Facing Slope (NFS) and the South Facing Slope (SFS) as well as the canyon floor (Ellanskaya *et al.* 1997). The diversity of cryptogamic plants and fungi has been studied, in the context of a research program of biodiversity across phylogeny, at the Evolution Canyon microsite. The opposite slopes of Evolution Canyon display dramatic biotic contrasts due to higher (up to 300%) solar radiation on the SFS which is warmer, drier, and climatically more fluctuating than the NFS (Volz *et al.* 1997).

To date, the main focus has been on studies of biodiversity of different groups of higher plants and animals (land snails, scorpions, beetles, ants, fruit flies, reptiles, birds, and rodents) at Evolution Canyon (Nevo 1995). The "tropical" SFS harbors, across phylogeny of plants and animals, an abundance of Asian and African taxa, and is richer in genetic diversity than the "temperate" NFS habitats.

Initial studies of soil micro-fungi in Israel indicated a diverse group of species in rural and urban areas (Volz *et al.* 1992). Dominant species belonged to the genera *Chrysosporium, Gliocladium, Memnoiella*, and *Emericellopsis*. Mount Carmel as well as Akko Park and Kiryat Shoma Park contained high numbers of diverse species in soil samples (Volz *et al.* 1997). Both pathogenic and nonpathogenic species were recovered (Volz & Wasser 1994). Pathogenic species included *Microsporium gypseum, M. ferrugineum*, and *Trichophyton terrestre*. Further investigations revealed frequency of isolation reflected land use habitats unchanged for centuries with well established micro-fungal populations (Volz & Wasser 1995). In Evolution Canyon, species richness was much higher on the NFS than on the SFS. *Aphanocladium album, Cladosporium tenuissimum, Sepedomium chrysospermum, Ulocladium botrytis, Fusarium acuminatum*, and *Phoma exigua* were found on the valley floor and not on either slope.

A combined research program initiative to enhance the knowledge on diversity of mosses, algae, and fungi (including lichens and lichenaceous fungi) was started at Evolution Canyon. Soil micro-fungi studies for this research were focused on interslope systematic, phenotypic, and genotypic diversity comparisons of representative taxa. Particular attention is also being paid to: (a) species present on both slopes; (b) species differing quantitatively on both slopes; and (c) species restricted to one of the slopes (Wasser *et al.* 1995). Interslope divergence of species composition derives from the different solar radiation and results in dramatic physical and biotic differentiation over a very short geographic distance (Lavie *et al.* 1993; Nevo 1995; Wasser *et al.* 1995). The current investigation directs attention to sporulation abundance influenced by micro-fungal species isolation according to the N or S facing slope of the canyon.

MATERIALS AND METHODS

Pure cultures of the test micro-fungal species from the North Facing Slope (NFS) and South Facing Slope (SFS) of Evolution Canyon, Lower Nahal Oren, Mt. Carmel, Haifa, Israel were collected and maintained from soil sampling methods previously described (Kirk & Ansell 1992; Singleton *et al.* 1992; Volz *et al.* 1997). The NFS and SFS isolates included the following species: *Alternaria alternata* (Fries) Keissler (Ellis 1971), *Aspergillus niger* van Tieghm (Raper & Fennel 1965), *Fusarium sambucinum* Fuckel, *Fusarium solani* (Martius) Saccardo (Booth 1971), *Humicola grisea* Traeen (Barron 1968), *Mucor hiemalis* Wehmer (O'Donnell 1979), *Oidiodendron cerealis* (Thuemen) Barron (Barnett & Hunter 1972), *Sordaria fimicola* (Roberge) Cesati & De Notaris (Ellis 1971; Ellis 1976), *Stachybotrys chartarum* (Ehrenberg) Huges (Watanabe 1993), *Staphylotrichum coccosporum* Nicot & Meyer (Gilman 1966), and *Ulocladium consortiale* (Thuemen) Simmons (Watanabe 1993).

Colony spore production of each species isolated from both North and South slopes of Evolution Canyon was monitored for a period of one month for variation in development according to canyon exposure. The Lower Nahal Oren microsite is located at 32° 43′ N 34° 58′ E. The Plio-Pleistocene canyon is about 3-5 million years

old, and the two opposite slopes of Nahal Oren share the same geology of limestone and dolomite of the upper Cenomanian geological formation. The two slopes are separated by about 500 m at the canyon top. The opposite canyon slopes show biotic contrasts in species density and diversity due to higher solar radiation on the SFS, which is warmer, drier, and climatically more fluctuating than the NFS (Gutterman & Nevo 1994). Canyons with contrasting south and north slopes are excellent testing models in nature for evolutionary theory and its multiple predictions across life (Wasser *et al.* 1995).

RESULTS

The margin density of conidiophore production for *Aspergillus niger* varied according to NFS and SFS (Figures 1 and 2) isolates from Evolution Canyon. The SFS isolate produced abundant conidiophore and spore production with all carbon sources except for melibiose. No margin conidiophore production was present in the NFS isolate with glucose, citrate, sucrose, rhamnose, inulin, and maltose. Only d-arabinose and melibiose produced equal conidiophore production with both the NFS and SFS isolate. The SFS isolate from worst to best conidiophore production was citrate, lactose, maltose, sucrose, rhamnose, melibiose, d-arabinose, l-arabinose, inulin, and glucose, respectively (Table 1).

For *Mucor hiemalis*, the SFS isolate produced a greater abundance of margin spore production in comparison to the NFS isolate with all sugars except for glucose, sucrose, rhamnose, and maltose. The worst to best carbon source was citrate, d-arabinose, maltose, rhamnose, glucose, and sucrose, respectively. The worst to best sugar for the SFS isolate included lactose, maltose, sucrose, inulin, citrate, d-arabinose, melibiose, rhamnose, glucose, and l-arabinose, respectively.

The asexual spore production for some test micro-fungal isolates indicated that the NFS and SFS were less clearly separated according to site of isolation and growth on carbon source. *Fusarium sambucinum* NFS and SFS isolates produced margin spore and sclerotia equally well on all carbon sources. *Humicola grisea* NFS and SFS isolates produced margin spores on all sugars, while other micro-fungal isolates from the NFS and SFS varied slightly according to species with respect to the NFS and SFS isolates and corresponding carbon sources (Table 2).

The *Mucor hiemalis* NFS isolate initiated zygospore production on all carbon sources earlier than the SFS isolate except for maltose, melibiose, and l-arabinose. With these three carbon sources, zygospore production initiated at the same time for both the NFS and SFS isolates. Sucrose and d-arabinose zygospore production initiated three days earlier for the NFS isolate compared to the SFS isolate. With all other carbon sources, zygospores were present two days earlier in the NFS colonies compared to the SFS colonies (Table 3).

Figure 1. *Aspergillus niger* NFS isolate colony spore production at one month growth at room temperature on SMA. 270x.

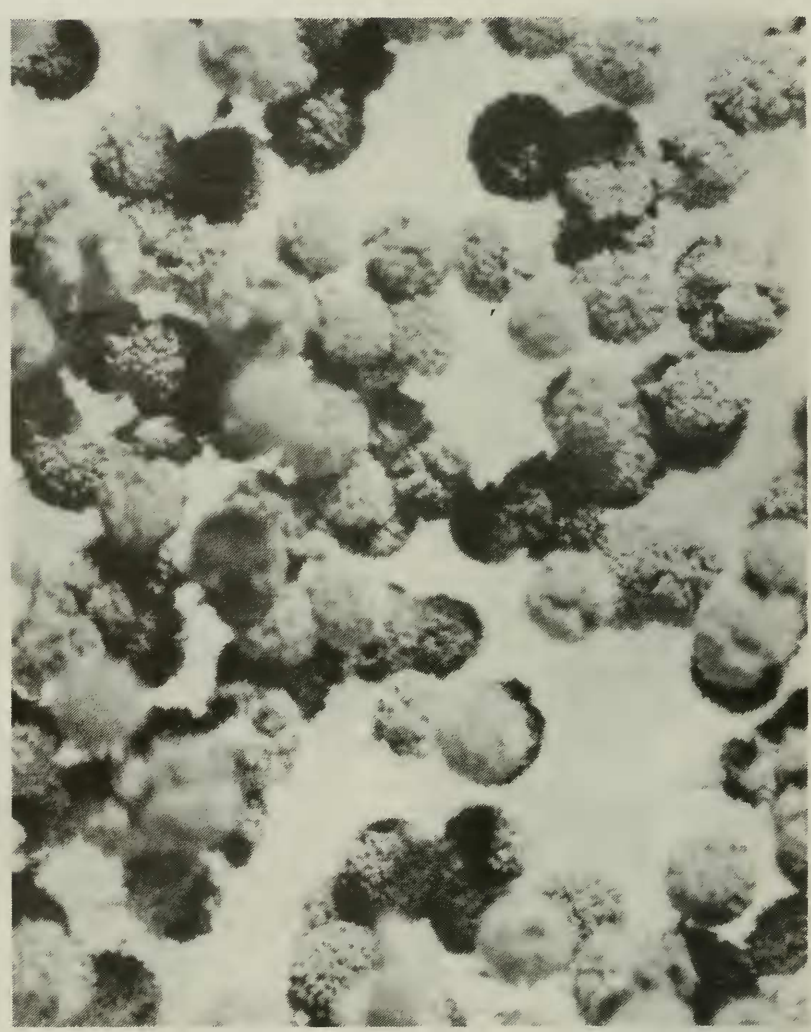

Figure 2. *Aspergillus niger* SFS isolate colony spore production at one month growth at room temperature on SMA. 270x.

Table 1. Spore production of micro-fungi from the NFS and SFS of Evolution Canyon when grown on selected carbon sources. dara = d-arabinose; mel = melibiose; glu = glucose; lara = l-arabinose; lac = lactose; cit = citrate; suc = sucrose; rha = rhamnose; inu = inulin; mal = maltose; + = yes; - = no; s = sclerotia; N = north; S = south; A. = *Alternaria*; F. = *Fusarium*; H. = *Humicola*; O. = *Oidiodendron*; S. = *Sordaria*; St. = *Stachybotrys*; Sa. = *Staphylotrichum*; and U. = *Ulocladium*.

sugar / **isolate**	dara	mel	glu	lara	lac	cit	suc	rha	inu	mal
A. alternata S	-	+	+	-	+	+	+	+	+	+
A. alternata N	-	-	+	-	+	+	+	-	-	-
F. sambucinum S	+	+	+	+	+	-	+	+	+	+
F. sambucinum N	+s	+s	+s	+s	+s	-s	+s	+s	+s	+s
H. grisea S	+	+	+	+	+	+	+	+	+	+
H. grisea N	+	+	+	+	+	+	+	+	+	+
O. cerealis S	+	+	+	+	+	+	+	+	+	+
O. cerealis N	+	+	+	+	+	+	+	+	+	+
S. fimicola S	+	+	+	+	-	-	+	+	+	+
S. fimicola N	-	-	-	-	-	-	-	-	-	-
St. chartarum S	+	-	+	+	+	-	+	+	+	+
St. chartarum N	+	-	+	+	-	-	+	+	-	+
Sa. coccosporum S	-	-	-	-	-	-	-	-	-	-
Sa. coccosporum N	+	+	+	+	+	+	+	+	+	+
U. consortiale S	+	+	+	+	+	+	+	+	+	+
U. consortiale N	+	-	+	-	+	-	+	-	-	+

Table 2. Spore density of *Aspergillus niger* and *Mucor hiemalis* from the NFS and SFS of Evolution Canyon when grown on selected carbon sources. dara = d-arabinose; mel = melibiose; glu = glucose; lara = l-arabinose; lac = lactose; cit = citrate; suc = sucrose; rha = rhamnose; inu = inulin; mal = maltose; N = north; S = south; A. = *Aspergillus*; M. = *Mucor*.

sugar / **isolate**	dara	mel	glu	lara	lac	cit	suc	rha	inu	mal
A. niger S	55	40	88	59	3	1	21	29	62	10
A. niger N	40	43	-	1	1	-	-	-	-	-
M. hiemalis S	44	46	110	130	17	35	29	60	30	20
M. hiemalis N	17	26	200	40	15	16	200	100	25	90

Table 3. Zygospore production of *Mucor hiemalis* from the NFS and SFS of Evolution Canyon when grown on selected carbon sources. d-ara = d-arabinose; mel = melibiose; glu = glucose; l-ara = l-arabinose; lac = lactose; cit = citrate; suc = sucrose; rha = rhamnose; inu = inulin; mal = maltose; + = yes; - = no.

Day	1	2	3	4	5	6	7	8
South								
d-ara	-	-	-	-	-	+	+	+
mel	-	-	-	-	+	+	+	+
glu	-	-	-	-	-	+	+	+
l-ara	-	-	-	-	+	+	+	+
lac	-	-	-	-	+	+	+	+
cit	-	-	-	-	+	+	+	+
suc	-	-	-	-	+	+	+	+
rha	-	-	-	-	+	+	+	+
inu	-	-	-	-	+	+	+	+
mal	-	-	-	-	+	+	+	+
North								
d-ara	-	-	+	+	+	+	+	+
mel	-	-	-	-	+	+	+	+
glu	-	-	+	+	+	+	+	+
l-ara	-	-	-	-	+	+	+	+
lac	-	-	+	+	+	+	+	+
cit	-	-	+	+	+	+	+	+
suc	-	+	+	+	+	+	+	+
rha	-	-	+	+	+	+	+	+
inu	-	-	+	+	+	+	+	+
mal	-	-	-	+	+	+	+	+

DISCUSSION

In a previous study, the SFS isolates of Evolution Canyon exhibited greater change in colony morphology than the NFS isolates of the same species. For example, the NFS *Alternaria alternata* isolate produced a colony of dense luxuriant growth, grey to tan to black coloration zones, very cottony hyphae, zonate growth of bands dark brown to tan, a flat margin, and a reverse colony which was dark brown to black. The SFS isolate of *Alternaria alternata* appeared to be quite weak, was less pigmented, and had very little colony development. Likewise, for all other micro-fungal species examined, colony morphology and hyphal growth dynamics reflected the area of isolation according to the N or S facing slope of the canyon (Rosenzweig & Volz 1998).

Fusarium sambucinum is a common isolate found in soil habitats (Watanabe 1993). Similarly, the isolate was frequently found on both the NFS and SFS of Evolution Canyon. Members of the *Fusarium* genus easily and freely produce an abundance of micro-conidia of various sizes as well as various cell number per conidium (Gilman 1966). Both *F. sambucinum* NFS and SFS isolates easily produced conidia in the actively growing areas of all colonies when grown on each of the ten test carbon sources, including the control carbon source maltose. Similar to *F. sambucinum*, other micro-fungi found in Evolution Canyon soil also produced asexual conidial spores on all carbon sources used in the study. *Humicola grisea, Mucor hiemalis, Oidiodendron cerealis*, and *Sordaria fimicola* NFS and SFS isolates produced spores on all ten test carbon sources in the young actively growing peripheral margin zone of all colonies. No inhibition of conidial production was noted between isolates found on opposing slopes of the canyon or when the isolates were grown on any of the ten carbon sources. Other canyon micro-fungi yielded varied results, influenced by their slope isolation locality according to individual species. The sporulation was also reflective of the species capability to withstand damaging UV radiation.

Alternaria alternata, a dematiaceous micro-fungal species, freely sporulated when isolated from the SFS of the canyon and grown on most carbon sources. Sporulation occurs when a colony is environmentally challenged and exhibits colony stress. No spores were produced in the colony margin of either the NFS or SFS isolate when grown on d-arabinose or l-arabinose indicating inhibitory effects toward sporulation when grown in the presence of these sugars. Likewise, more frequently *Sordaria fimicola* SFS isolate, *Stachybotrys chartarum* SFS isolate, and *Ulocladium consortiale* SFS isolate generally produced spores on the test carbon sources. Often spores did not form in the actively growing colony margin when the NFS counterpart isolate of the same species was isolated from the opposite more environmentally solar protected canyon slope. Only maltose, a good carbon source for growth and used as the control sugar, initiated spore production for both *Sordaria fimicola* NFS and SFS isolates while no spores were produced when *Sordaria* isolates were grown on citrate, a poor source for growth rates for many micro-fungi.

Conversely, *Staphylotrichum coccosporum* NFS isolate readily produced spores on all ten test carbon sources while no spores were produced in the colony actively growing hyphal zone with the SFS isolate of the same species on the ten carbon

species, unanimously opposite to the sporulation patterns of all other canyon micro-fungal species. Environmental stress on the south canyon slope may have been too great for spore production of *S. coccosporum* while the NFS isolate was able to grow and sporulate normally on all carbon sources when subjected to less environmental stress as found on the north slope of the canyon.

Mucor hiemalis, in addition to asexual sporangiospores, readily produced the sexual heterothallic zygospores as well, unlike the remaining deuteromycetous micro-fungi canyon species included in the study capable of forming only conidial spore forms. Sporangiospore production appeared on the second day when the *Mucor hiemalis* NFS isolate colony grew on media with sucrose as the carbon source. NFS isolate colonies produced spores on day 3 or 4 incubation on all other carbon sources. The SFS *M. hiemalis* isolate colonies formed sporangiospores significantly later on the selected carbon sources. For the SFS isolate, spore production occurred on day 5 for most carbon sources, and day 6 for d-arabinose and glucose. Zygospore formation developed on day 2 of incubation using maltose as the carbon source. Maltose even enhanced zygospore production, a process that generally requires more than two days from the time of inoculation of the isolate on the test agar media to the presence of mature zygospores. *Mucor hiemalis* SFS isolate demonstrated enhanced colony development on maltose and inhibited or delayed zygospore development on all other test carbon sources. The delay in zygospore production, except when maltose was used in the agar medium was evident with the SFS isolate, indicating environmental stress on the south canyon slope for the *M. hiemalis* micro-fungal species. Normal development and spore production occurred with the *M. hiemalis* NFS isolate, producing little to no direct light effects for this fungus when grown on the north canyon slope.

Diversity of growth rates, sporulation, and colony development between isolates of the same micro-fungal species collected from the NFS and SFS of Evolution Canyon reflects the diversity of habitats between the opposing slopes separated by only 500 m at the canyon top. The south slope is quite barren, arid, and devoid of vegetation, while the north slope is covered with dense growth of vegetation (Wasser *et al.* 1995). The south slope received up to 300% higher solar radiation in contrast to the cooler and more moist north slope, thus much higher and more intense levels of UV light including the more mutagenic wavelengths of 254, 280, and 300 nm band widths.

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